Mid-Infrared Spectroscopy for Juice Authentication –

Improvement of Modeling Power for Juice Differentiation by Analyzing Signature-like Phenolic Spectra

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Abstract

Determination of food authenticity is an important issue in food safety and quality control. Mid-infrared spectroscopy provides rapid chemical profiling of agricultural products and could become an effective tool for authentication when coupled to chemometrics. This study developed a simple protocol for classifying commercial juices using attenuated total reflectance infrared spectroscopy, and sought for improvement of modeling power by analyzing the taxonomic compounds in the phenol-rich fraction. Spectra from 52 juices together with their extracted sugar-rich and phenol-rich fractions were obtained to construct multivariate models (HCA and SIMCA) for pattern recognition analysis and prediction. Spectra of the sugar-rich fraction, comprising primarily of sugars and simple acids, almost superimposed the whole juice spectra. Solid phase extraction enriched phenol compounds and provided signature-like spectral information that substantially improved the SIMCA modeling power over the whole juice or sugar-rich fraction models. Zero-percent misclassification was achieved by the phenol-rich fraction model at commodity and manufacturer level, where as the whole juice model was only feasible for commodity level differentiation. HCA successfully recognized the natural grouping of juices based on ingredients similarity and revealed that the phenol fraction contained sufficient information to differentiate among the same type of juice from different manufacturers. The infrared technique assisted by phenol fractionation and chemometrics provided a promising analytical method for the assurance of juice quality and authenticity.

Introduction

The source of raw fruit material is essential to the steadily high quality of the finished juice/wine product and to the compliance with labeling. However, partial replacement of high cost ingredients with lower grade or cheaper substitutes can be very attractive and lucrative for a fruit supplier. Raw fruit materials with different varieties and geographical origins could have greatly different prices yet it is hard to differentiate the source. These factors have underlined the need of rapid, reliable, easy-to-use and cost-effective techniques for the juice/wine industry and regulatory agencies to effectively check the authenticity of the incoming fruit material. Traditional chromatography methods are costly, time consuming, and dependant on non-universal marker compounds like sorbitol (1) and certain anthocyanins (2).

The infrared (IR) spectra (4000-700 cm^{-1}) reflect the total biochemical composition of a sample and provide structural information based on chemical functional groups, thus variation in the chemical composition attributed to varietal or geographical origin difference may be elucidated through chemometric analysis. Despite that, only a few studies have focused on authentication of fruit product with IR (3, 4, 5), all done with intact product.

Anthocyanins and other phenols in grapes, berries and wines had been reported to be signature compounds for taxonomic purpose, but their IR signal could be masked by other dominant compounds in the intact juices such as sugars and acids. Therefore we hypothesized that we may find improved prediction power using the phenol-rich fraction as compared to using the intact juice.

The ultimate goal of our research is to provide the juice/wine industry and regulatory agencies a rapid, high-throughput and low cost analysis of juice authenticity and quality. In this study we developed a robust method to evaluate intact fruit juices as well as their sugar-rich and phenol-rich fractions for differentiation of juices and identification of potential adulterants.

Results

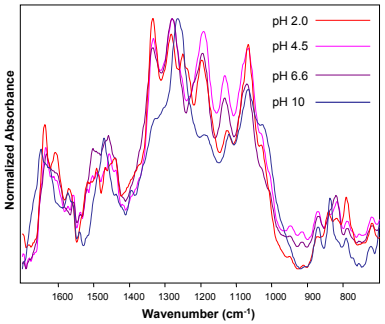


Figure 2. The effect of pH on the IR profile of a purified berry anthocyanin mixture.

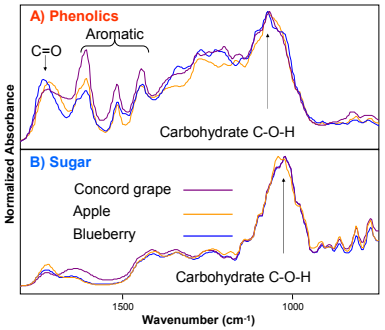
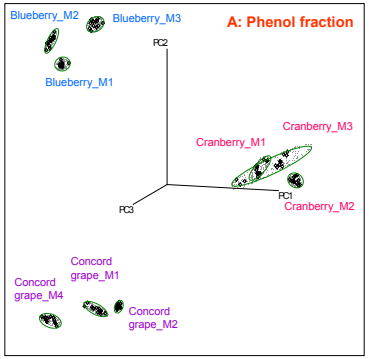


Figure 3. Mid-IR spectra (1800-750 cm^{-1}) of the phenol-rich fraction (A) and the sugar-rich fraction (B) of blueberry, concord grape, and apple juices. The absorbance has been normalized to the most intense band for each spectrum.



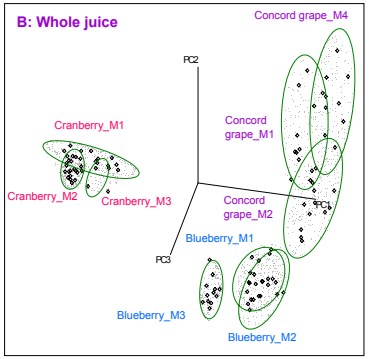


Figure 4. Juice clustering in SIMCA 3D class projections plots. Three axes represent the first 3 PCs extracted. Only one angle is shown to illustrate the clusters. The ovals represent the 95% confidence intervals of each cluster.

Table 1. Prediction of validation set juice identities by the SIMCA calibration model. The "next best" prediction usually occurs when the confidence intervals of clusters approximate each other or even overlap.

Juices [1]	Prediction based on different fractions [2]					
	Whole Juice					Phenol Fraction
	Best	Next Best				Best
		1	2	3	4	
Concord grape_M4 [3]	Concord grape_M2	Concord grape_M1	Blueberry_M2	Concord grape_M4	Blueberry_M1	Concord grape_M4
	Concord grape_M2	Concord grape_M1	Blueberry_M2	Concord grape_M4	Blueberry_M1	Concord grape_M4
	Concord grape_M2	Concord grape_M1	Blueberry_M2	Concord grape_M4	Blueberry_M1	Concord grape_M4
Concord grape_M5	Concord grape_M1	Concord grape_M4	Concord grape_M2	Blueberry_M2	N/A [4]	Concord grape_M1
	Concord grape_M1	Concord grape_M4	Concord grape_M2	Blueberry_M2	Blueberry_M1	Concord grape_M1
	Concord grape_M1	Concord grape_M4	Concord grape_M2	Blueberry_M2	N/A	Concord grape_M1
Grape_blend (15%)	Blueberry_M1	N/A	N/A	N/A	N/A	N/A
	N/A	N/A	N/A	N/A	N/A	N/A
	Blueberry_M1	N/A	N/A	N/A	N/A	N/A
Grape_blend (100%)	Concord grape_M2	Concord grape_M4	Concord grape_M1	Blueberry_M2	N/A	N/A
	Concord grape_M2	Concord grape_M1	Concord grape_M4	N/A	N/A	N/A
	Concord grape_M2	Concord grape_M1	Blueberry_M2	Concord grape_M4	N/A	N/A

[1] Four new juices for external validation. [2] Each juice in the validation set was predicted with 3 replicated spectra. [3] M followed by a number means a manufacturer. [4] N/A means no matching to established category.

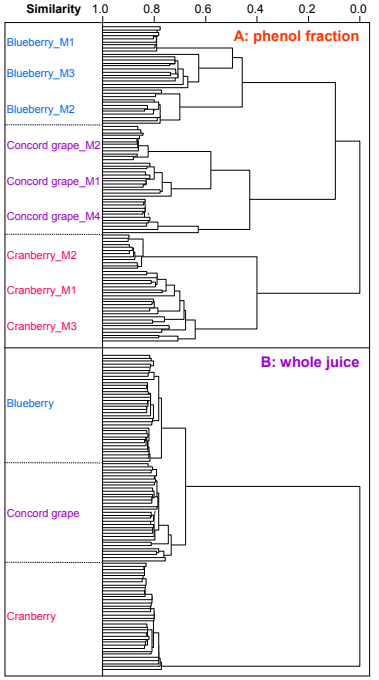


Figure 5. HCA dendrograms showing similarity among 108 juice IR spectra from each fraction – phenol-rich fraction (A) and the whole juice (B). The numbers on the X axis represent the similarity among samples. Similarity of 0 means the largest distance in the data set, and similarity of 1 means identical samples.

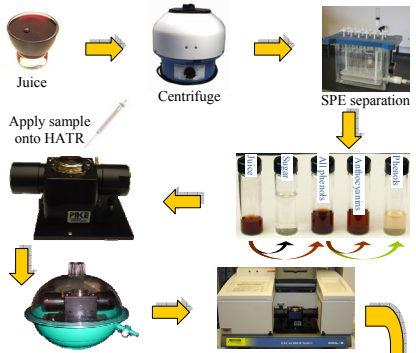


Figure 1. Flow chart of juice fractionation and IR spectral data acquisition.

Materials and Methods

Juice samples. Twelve different juices were obtained from local grocery stores to test the feasibility of using FTIR to recognize subtle compositional differences among different juices. Afterwards, cranberry, blueberry and Concord grape juices each manufactured by three companies and four different batches from each company (a total of 36 samples), were obtained to evaluate difference caused by origin/manufacturer and processing conditions. Four additional juices were used to validate the calibration model.

Sample preparation. Juice samples were centrifuged at 3,000 rpm for 10 min to remove non-soluble solids, and then prepared as 3 fractions (whole juice, sugars and phenolic fractions) with 3 replications.

The whole juice fraction was made by mixing 1 volume of supernatant juice with 2 volume of acidified distilled (DD) water (0.1% HCl).

The sugar-rich fraction and phenol-rich fraction were prepared using solid-phase extraction (SPE) (6). Using the protocol developed in our lab, the phenol-rich fraction was further separated into anthocyanin fraction and non-anthocyanin fraction to evaluate the role of anthocyanins (Figure 1).

Standards (all ACS reagent grade) of sucrose, glucose (Acros, NJ), fructose (Sigma, St. Louis, MO), gallic acid (MP Biomedicals, Aurora, OH) and a representative anthocyanin cyanidin-3-glucoside (Polyphenols, Norway) were also analyzed to aid in the interpretation of the juice spectra.

Spectra acquisition. A Digilab Excalibur 3500 FTIR spectrometer (Digilab, Randolph, MA) equipped with a horizontal attenuated total reflectance (HATR) accessory (Pike Technologies, Madison, WI) was used to collect all spectra. Ten µL of aqueous sample or 15 µL of aqueous methanol sample was deposited onto the HATR crystal via a syringe, and dried in a vacuum chamber for 5 min. Each juice fraction was applied 3 times for repeated measurement. Spectra were collected over the frequency region from 4000-700 cm-1.

Chemometrics. The spectra were imported as spreadsheet into a multivariate statistics program Pirouette 3.11 (Infometrix Inc., Woodinville, WA). Second derivative and normalization were performed on all data before the supervised clustering analysis- Principle Component Analysis (PCA) and Soft Independent Modeling of Class Analogy (SIMCA) analysis- and the unsupervised clustering analysis - Hierarchical Cluster Analysis (HCA).

Discussion and Conclusions

The phenol-rich fraction spectra was greatly affected by pH (Figure 2). As pH dropped below 2, the spectra became stable. Therefore we decided to use acidified solvents for phenol-rich fraction. Anthocyanins, flavonoids, cinnamic acids, and flavor compounds were distinctive for each commodity, resulting in varied spectra (Figure 3A).

The whole juice spectra major bands were almost identical to those in the sugar-rich fraction, which contained primarily sugars and the non-phenolic acids such as tartaric, malic and citric acids. Statistic models also demonstrated minute difference between the whole juice and sugar-rich fraction spectra, showing that useful phenol compounds signals could be masked by major juice components in juice.

Peak assignments were made for each fraction IR spectra. In the phenol-rich fraction (Figure 3A), the broad band in the 1160-1000 cm^{-1} region was assigned to the glycosylation of phenolics, and partly to aromatic C-O stretching. The peak at 1720 cm^{-1} was assigned to the carbonyl C=O stretching band of protonated carboxylic acids. In the sugar-rich fraction and whole juice, the broad band in the 1160-1000 cm^{-1} region was assigned to the C-O stretching vibration of the sugars (Figure 3B). The peak at 1710 cm^{-1} was assigned to the C=O stretching band of aldehyde/ketone groups in carbohydrates.

Feasibility of FTIR was shown by the successful separation of IR spectra from 12 different juices, with similar juices grouped closely. Potential adulterant, the apple juice, was separated far away from the high value juices. The results demonstrated feasibility of using FTIR to pickup subtle compositional differences among juices.

Multivariate analysis of juices from different batches and manufacturers

Clustering by SIMCA. Based on the **phenol-rich fraction** model (Figure 4A), 100% correct classification was achieved at the commodity level and manufacturer level, indicating the potential to differentiate varietal or processing condition difference. Neither of the juice blends was predicted as any of the pure juices in the model (Table 1), indicating the excellent prediction power of the phenol-rich fraction model. In contrast, based on the **whole juice model** (Figure 4B), juice blends were incorrectly assigned the identities of pure juices (Table 1). It is evident that the phenol-rich fraction model effectively improved the prediction power for unknown samples.

Clustering by HCA. HCA is an unsupervised clustering method used to visualize the natural grouping of the juice spectra. In the phenol-rich fraction dendrogram (Figure 5A), juices were grouped into naturally related clusters and even the manufacturer of juice was distinguished. **The phenol-rich fraction** also contained useful information to associate the juice blends to their ingredients (data not shown), suggesting the potential use of multivariate regression models to predict the concentration of juice ingredients. **The whole juice** dendrogram (Figure 5B) shows that 36 samples in the training dataset were grouped into 3 distinct classes, blueberry, Concord grape, and cranberry. However, the sensitivity was not high enough to differentiate at the manufacturer level within each type of juice.

In conclusion, an analytical method was developed to obtain reproducible spectra for juice authentication. Solid phase extracted phenol compounds provided signature-like spectral information that had improved modeling power over the regularly studied whole product spectra. The constructed statistic models demonstrated the potential to provide the juice/wine industry with a rapid and reliable tool for authenticating incoming materials and monitoring the finished product quality.

References

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